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Field Trial with the Entomopathogenic Fungus *Metarhizium anisopliae* var. *acridum* against Bands of the Grasshopper *Rhammatocerus schistocercoides* in Brazil

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The efficacy of a mycoinsecticide formulated in vegetable oil was tested in Brazil against the grasshopper Rhammatocerus schistocercoides. *A set of experiments was conducted in the Chapada dos Parecis region (Mato Grosso state), a permanent zone of outbreaks for this pest. Experiments were performed in zones of natural vegetation, against grasshopper bands in the third nymphal instar. Three nymphal bands were treated with a mycoinsecticide formulation based on conidia of the entomopathogenic fungus* Metarhizium anisopliae *var.* acridum (=M. flavoviride*), strain CG 423. Three non-treated bands were used as control. The application was made with the aid of a hand-held ULV sprayer adjusted to deliver 2 l of the formulation* ha^{-1}*, each containing* 1×10^{13} *conidia. Treatments were limited to the surface of the grasshopper bands and their immediate borders (5–10 m). The efficacy of the mycoinsecticide was evaluated through band survival after treatment (grasshopper numbers, surface, density, behaviour and daily movement of the band), allowing the insects to move freely in their natural environment. Insects were regularly surveyed and maintained in the laboratory, allowing estimates of the infection rate. Field and laboratory studies showed a clear effect of the product 10 days after treatment. At 14 days post-spraying, mortality caused by the mycoinsecticide in the field was approximately 88%.*

Keywords: *entomopathogenic fungus,* Metarhizium anisopliae *var.* acridum, Metarhizium flavoviride, *grasshopper,* Rhammatocerus schistocercoides, *oil formulation, field trial, Brazil*

INTRODUCTION

In the last decade, massive applications of the chemical insecticides fenitrothion and malathion were adopted to control grasshoppers in many countries. As a result of the

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combined effects of high cost of application and environmental contamination, there has been a significant increase in the development of methods to replace the use of chemical insecticides. One such method is the development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* var. *acridum* (Driver *et al*., 2000) (=*Metarhizium flavoviride*) as bioinsecticides. Application of *B. bassiana* formulated in oil ($2–5 \times 10^{13}$ conidia ha^{-1}) has provided significant reduction in grasshopper populations (Jaronski & Goettel, 1997; Inglis *et al*., 1997). Field applications of *M. anisopliae* var. *acridum* in Africa (isolated IMI 330189; Lomer *et al*., 1997) and Australia (F 1985 = ARSEF 324; Milner, 1997) achieved 50–80% control of grasshoppers. In some other cases, the level of control obtained was close to 100% (Lomer *et al*., 1999).

In some years, the grasshopper *Rhammatocerus schistocercoides* is a serious pest in Brazil, causing severe damage in rice, corn, sugarcane and native grasses (Lecoq & Pierozzi, 1995a, b; Lecoq *et al*., 1996; Miranda *et al*., 1996). Since 1983 there have been outbreaks causing crop losses in the cultivated areas of Mato Grosso and Rondônia states located between parallels 12° and 15° South and meridians 52° and 61° West (Lecoq, 1991). We report here on a field trial performed in Mato Grosso in November and December 1998 to evaluate the efficacy of a Brazilian isolate of *M. anisopliae* var. *acridum* against *R. schistocercoides* third instar nymphs in strictly natural conditions.

MATERIALS AND METHODS

Experimental Area, the Grasshopper and its Biotopes

The study was conducted in central-western Mato Grosso, Campos de Júlio County, in Chapada dos Parecis, around ALCOMAT alcohol mill (14°16′S, 59°14′W), a typical *R. schistocercoides* permanent outbreak area (Lecoq & Pierozzi, 1994a; Miranda *et al*., 1996). The landscape in the ALCOMAT area is representative of the whole Chapada dos Parecis, being uniform with gently rolling ground, interlaced with a few rivers edged with well-developed gallery forests. The main plant communities are pure savannas and bushy or forested savannas, locally called 'campo', 'campo-cerrado' and 'cerrado', depending on the extent of the woody plant layer. The first two communities are natural biotopes for *R. schistocercoides* which can be divided into breeding biotopes, that are usually on sandy soils, and dry season dispersion/wandering biotopes, on heavier sand-clay soils (Miranda *et al*., 1996).

The climate of the region is characterized by an annual rainy season/dry season cycle (September–April/May–August). The mean yearly precipitation is about 200 cm. The mean monthly temperatures range from 20.3°C in July to 24°C in October, and are moderated by the land elevation (> 600 m) (data from Cuiabá Meteorological Service and from ALCOMAT synoptic meteorological station).

R. schistocercoides has only one generation a year. Grasshopper development is very slow (8–9 instars), from late October to late April, during the rainy season. The first adults appear by mid-April and remain immature until late August. Sexual maturation is achieved in one month and egg laying occurs from late September to late October (usually no more than three clutches per female). The behaviour of the species is very gregarious: grasshoppers form bands (usually tens to thousands m^{-2}, up to 5000 m^{-2}; in some areas of the band density may reach 30 000 m^{-2} and adults form swarms (thousands of square metres to several hectares) that partially leave the breeding biotope areas to wander throughout the natural vegetation (Lecoq & Pierozzi, 1994a;b; Launois-Luong & Lecoq, 1996; Miranda *et al*., 1996).

Experiments were conducted at the beginning of the rainy season, from 20 November to 7 December, 1998, in breeding biotopes, in areas of natural vegetation frequently burnt during the preceding dry season. The grass layer was green (95% of total biomass), rather low (30–40 cm) and of its cover was generally high (> 80%). The bush and tree layer was also low (50–250 cm) and the extent of its cover very small (< 1–15%).

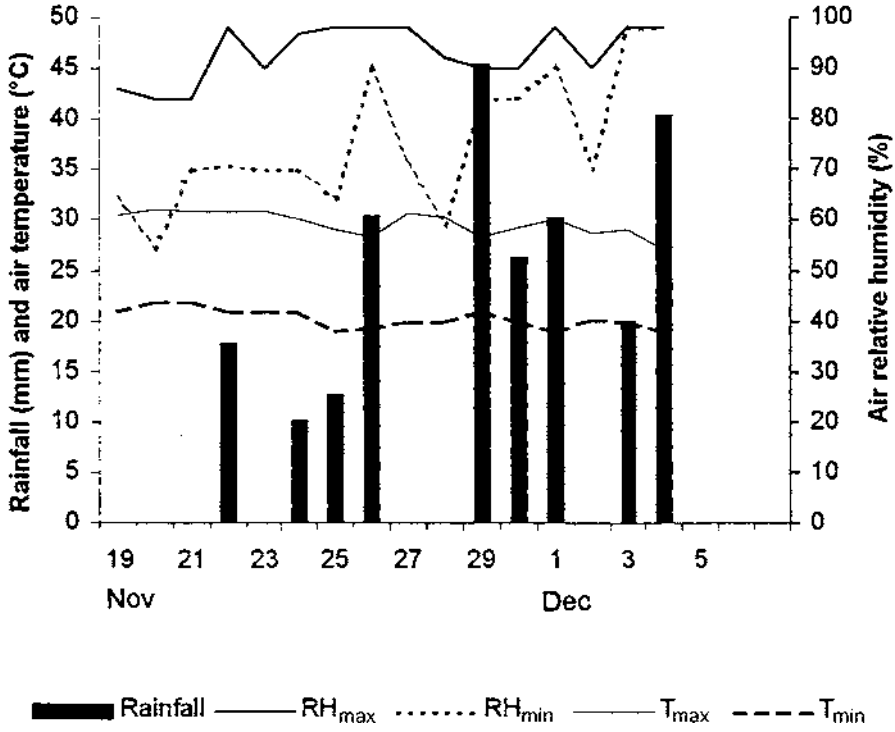

FIGURE 1. Meteorological conditions during the observation period.

TABLE 1. Meteorological conditions during treatment of *R. schistocercoides* hopper bands with *M. anisopliae* var. *acridum*

Bands	F1		F2		F3	
	Beginning	End	Beginning	End	Beginning	End
Time	16:45	18:00	15:40	16:40	18:15	19:00
Temperature (°C)	27.5	26.3	29.5	29.2	25.5	25.0
Relative humidity (%)	72	85	60	63	85	87
Wind direction (°)	250	70	260	260	140	140
Wind power (ms^{-1}) (average)	1.2		2.3		0.6	
Cloud cover	10/10		10/10		10/10	

Figure 1 shows the weather patterns during the experimental period. Temperature, relative humidity, cloudcover, wind direction and speed were recorded regularly during treatment (Table 1). Spraying was performed at 1 m height, with a mean operating speed of 1 m s^{-1}. The wind direction and mean force were noted at each swath, along with the spraying time to check the real flow rate of the nozzle.

At the beginning of the experiments, many *R. schistocercoides* grasshopper bands of third instar nymphs were present in the area. Seven grasshopper bands were chosen in natural *campo-cerrado* vegetation zones. The experimental area was systematically scouted according to a regular itinerary, to pinpoint bands that could be used in the experiment. The aim was also to assess their distribution, density and the probability of merging with neighbouring bands. The bands chosen for the tests were located quite far from each other to avoid

merging which, however, was unavoidable in some cases. They were also located quite far from any road to avoid a change in band behaviour and the crushing of hoppers by vehicles.

Mycopesticide Formulation

The strain CG 423 of *M. anisopliae* var. *acridum* used in this study was originally isolated from the grasshopper *Schistocerca pallens* (Moreira *et al.*, 1996). Conidia were mass produced on rice, according to Magalhães and Frazão (1996). Briefly, cooked par-boiled rice (100 g) containing 90% water, sterilized in autoclavable plastic bags (2 l) was inoculated with 5 ml of a fungal suspension (10^7 conidia m^{-1}) and kept in a room at 28ºC, 12 h photophase. Conidia were harvested 10 days post-inoculation by washing the substrate with kerosene. For field application, conidial concentration was adjusted to 1×10^{10} conidia per ml of suspension (50% kerosene:50% soybean oil).

Field Trials

A Micro Ulva + apparatus for ULV spraying, powered by six 1.5 V batteries, was used for the spray treatments. The sprayer was fitted with a red nozzle which enabled a flow rate of approximately 60 ml min^{-1}.

The treatment dose was 2×10^{13} conidia/ha, with 2 l of formulation applied, and spraying was limited to the area covered by each grasshopper band and its immediate surroundings (5–10 m). Three grasshopper bands were used as control (C1, C2 and C3). Three other bands were treated with the mycoinsecticide in the afternoon (F1, F2 and F3; Table 2). Conidial viability of > 90% in the oil formulation was determined immediately after application, according to methodology described by Magalhães *et al.* (1997).

An additional band was ULV-treated with a formulation containing conidia of *M. anisopliae* var. *acridum* (equivalent to 3×10^{13} conidia ha^{-1}) mixed with a sub-lethal dose of fenitrothion (3 g a.i. ha^{-1}). Temperature and relative humidity at the beginning (18:00) and end (18:35) of application were 26.9–25.9ºC and 70–78%, respectively. Average wind speed was 1.1 m s^{-1}. Although viability tests showed the fungus to have low viability in this formulation (< 5% germination), we decided to consider this band in our study in order to verify the effect of a sublethal dosage of the chemical on *R. schistocercoides* nymphs and to estimate conidial coverage as follows.

To remove conidia deposited on the cuticle of insects, and, therefore, estimate an average conidial dose per insect, 97 nymphs from the plots treated with fenitrothion were collected immediately after application and added to glass vials containing pure kerosene. Four vials containing between 11 and 59 nymphs, were stored for one week until processing in the laboratory. Vials were agitated at 250 rpm for about 2 h. Conidial suspension was then transferred to glass centrifuge tubes. After centrifugation (10 000 rpm; 5 min), kerosene was poured out and conidia resuspended in kerosene to determine the concentration of the conidial suspension by hemocytometer counts.

TABLE 2. Main characteristics of the experiments carried out to assess the field efficacy of *M. anisopliae* var. *acridum* against *R. schistocercoides*

Hopper band	F1	F2	F3
Nozzle output (ml min^{-1})	60.1	58.2	59.7
Emission height (m)	1	1	1
Number of swaths	7	11	10
Space between swaths (m)	5	5	5
Total length covered by swaths (m)	420	440	600
Theoretic walking speed (ms^{-1})	1	1	1
Surface treated (m^2)	2100	2200	3000
Formulation volume applied (ml)	450	458	618
Formulation volume ha^{-1} (ml)	2143	2082	2060
Conidia dose ha^{-1} ($\times 10^{13}$)	2.143	2.082	2.060

Measurement of Efficacy

Monitoring grasshopper bands. Treatment efficacy was assessed by measuring band area (treated and control bands), density, and daily movement. This technique has already been used to monitor *R. schistocercoides* grasshopper bands for around 12 days (Lecoq *et al*., 1999), and for pesticide tests (Lecoq & Balança, 1998).

The grasshopper bands (control and treated) were located and monitored daily and characterized every 2 days. The following operations were undertaken according to a standardized procedure:

(1) Searching for the band according to its position in the previous day and signs of its movements (faeces, browsed vegetation). Analysis of these signs also enabled us to determine whether the band had divided or possibly merged with another band.
(2) Determining the position of the band with a GPS (Garmin 12 XL).
(3) Flagging the edges of the band (colored flags). To avoid disturbing the insects, the band area was measured the next day with the aid of a compass and tape measure, after the band had moved on.
(4) Visually assessing the density over each segment of the band area. As the densities were very high, we used an assessment method involving approximate density classes: 5000, 2500, 1000, 500, 250, 100 and < 100 grasshoppers m^{-2}. Density gradients within the band were also evaluated by moving through the band several times from the front to the tail. Photographs were also taken of each band. The real density corresponding to visual assessments was checked later by analyzing these photographs.
(5) Collecting grasshopper specimens to determine their developmental stage.
(6) Measuring the distance travelled in the previous day.
(7) Monitoring the area around the band to detect bands that could merge or be misidentified as being part of the studied band.

To avoid disturbing the grasshoppers and facilitate the observations, grasshopper band scouting and assessment were performed daily by one observer, at the same time, from 07:00 h to 09:00 h, when the grasshoppers were still relatively immobile at their overnight roosting site (Lecoq *et al*., 1999).

The grasshopper bands were mapped using the collected data. The lengths and orientation of the segments measured in the field were transformed into orthonormal coordinates and inserted in a geographic information system (Map Info). The outline of the band was plotted, together with the different density zones within the band. The band area was determined and the population in each density zone and the total band populations were calculated. To follow the progress of the band throughout the observation period, the successive positions of the band were noted on a map, together with changes in band shape, orientation and movement speed.

Monitoring of Caged Insects

Fourteen hours following field application of the fungus, 50 insects of each band (=replicate), were collected and maintained in cages with wooden frames and nylon screen. The procedure was repeated three and seven days post-application. Cages were kept in an improvised laboratory at ALCOMAT. Insects were fed native grass (*Andropogon selloanus* Hackel) amended with cereal flakes. Cleaning and food renewal were performed every other day. Mortality was assessed daily and dead nymphs put inside wet chambers to confirm the cause of death. T-tests were run in order to compare treated and non-treated caged insects.

RESULTS

Evolution of Grasshopper Bands

Grasshopper development. At the time of treatment (20 November 1998), grasshopper

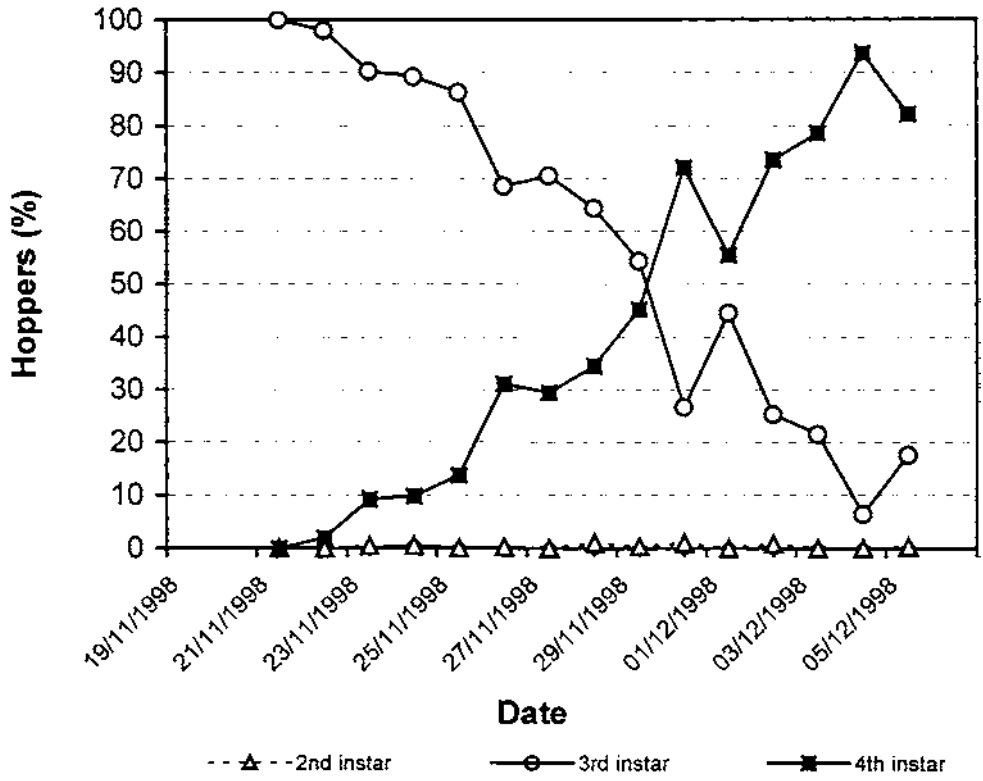

FIGURE 2. Grasshopper band development during the experimental period (average for 6 bands).

populations were all third instars. They gradually moulted into fourth instar over the next 15 days. Most moulting occurred between 26 and 30 November. At the end of the study, approximately 94% of nymphs of all bands were in the fourth instar (Figure 2). Rates of grasshopper development were identical in treated and control bands.

Band area. The mean area of treated bands increased and some differences in band patterns were noted. Band F1 increased from 100 m^2 at the beginning of the test period to 215 m^2 on 28 November, and then divided into two bands, which were initially small and dense, but then became less dense as they expanded. Band F3 increased regularly in size, from 315 m^2 to 1245 m^2, although the population decreased over time. Finally, the area of band F2 also increased from 146 m^2 to around 800 m^2, prior to merging with another band between 30 November and 2 December.

The areas of the control bands were around 200 m^2 for bands C1 and C2 and almost 700 m^2 for band C3 (Figure 3). The areas of bands C1 and C2 increased regularly (especially from 29 November to 1 December, during the moult) to reach a size of 600–700 m^2 on 1 December. Band C3 also increased in size to around 1000 m^2 on 27 November. It then divided into two bands between 27 and 29 November. For practical reasons, we only monitored one of these two new bands thereafter; it increased in size from 400 m^2 to around 800 m^2 at the end of the observation period.

Band movement. Daily band movements ranged from 2 to 70 m, with a mean of 22.7 m. Control and treated bands moved 24.4 and 19.6 m on average, respectively. Throughout the

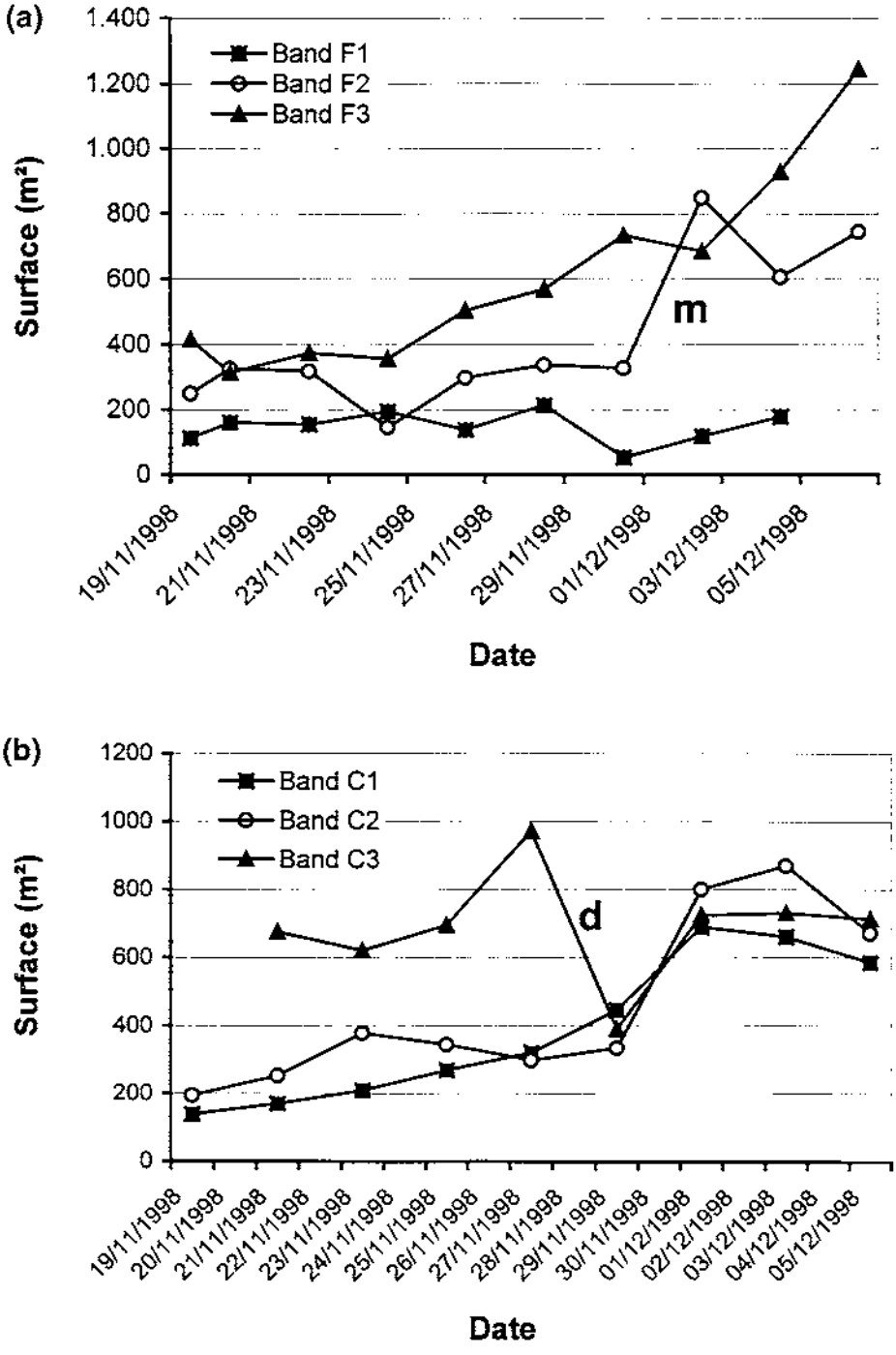

FIGURE 3. Evolution of grasshopper bands size during the experimental period: (a) treated and (b) untreated; d, division of band C3; m, merging of band F2 with another band; mycoinsecticide sprayed on 20 November 1998.

test period, the daily movements were 19.5, 22.6, 16.8, 17.6, 22.3 and 33.4 m for bands F1, F2, F3, C1, C2 and C3, respectively. During the moult period, the extent of the movements decreased progressively for all bands. It varied from around 35 m at the beginning of the test to around 10 m day^{-1} at the end of the moulting period. By 6 December, most hoppers had moulted into fourth instar, but their integuments had not yet hardened nor had they recovered their full movement potential. There were no marked differences between treated and control bands.

Band divisions and mergers. The bands were carefully monitored to detect division and merge phenomena, which occurred on several occasions. Several bands divided into two bands over the test period, either due to the effect of the mycopesticide and the subsequent marked decrease in the population (e.g. band F1 after 30 November and band F3 after 6 December), or due to the large size of the band (e.g. bands C3, F2 and C1 from 29, 30 November and 4 December, respectively). Except for band C3, the two bands formed after division were monitored and measured.

Conversely, band F2 probably merged with another band between 2 November and 4 December. Band C1 was also in the vicinity (± 15 m) of another band of the same size for 2 days, but it was moving in the opposite direction. Because of the high density of grasshoppers bands in the region, such mergers are certainly a common phenomenon during grasshopper development.

Grasshopper densities and population sizes: impact of the mycopesticide. In *R. schistocercoides* grasshopper bands, densities were maximal at the front of the band. Densities were around 5000 grasshoppers m^{-2} on average for third instar bands and around 2500 for fourth instar bands. Early in the morning, when the bands were clustered in the vegetation on the site where they had spent the night, average densities of more than 30 000 grasshoppers m^{-2} were noted. The density declined towards the tail of the band, where there were only a few dozen or hundreds of grasshoppers per square metre (Figures 4 and 5). At the peak of the moulting period, the band structure changed substantially. The band moved more slowly and the front was less clearly-defined. Maximum densities were noted around the middle of the band (cf. Figure 4, D + 8 and D + 10).

Population sizes of the studied bands ranged from approximately 500 000 to 2 000 000 grasshoppers (Figures 6 and 7). For treated bands, the mycopesticide had a clear effect, as shown by a marked decrease in grasshopper numbers (Figures 5 and 6A). The size of band F1 dropped from 547 000 grasshoppers during treatment to around 75 000 at 14 days post-spraying. Band F2 decreased from 1 070 000 grasshoppers to 292 000 at 12 days after fungal application (it then increased after merging with another band). Band F3 decreased from 624 000 grasshoppers to 118 000 at the end of the test period. At 14 days post-spraying, the mean mortality rate was estimated to be around 88% (mean for bands F1 and F3). For band F2, mortality was estimated before the merger (after 12 days) when it was 72.7%. Population reductions in the control bands were around 12–14% (Table 3).

For the control bands, the number of grasshoppers remained relatively stable over the test period (Figures 4 and 6(b)). The slight variations recorded seemed to be related mainly to the low accuracy of the assessment method. Estimated populations of band C1 and C2 were 550 000 and 850 000 grasshoppers, respectively. Over a period of approximately 8 days, the population of band C3 was estimated as 2 million grasshoppers. Between 28 and 30 November, the band divided into two bands, and was no longer considered in this study.

The effect of the mycopesticide was also clearly visible in the field. High numbers of grasshoppers exhibited unusual behaviour and were considered ill. At around 8 days post-treatment, we observed dead pinkish hoppers—a colour that signals fungal infection. As many dead and dying hoppers were still observed two days after terminating the experiment, it is quite likely that the fungal disease continued to spread and that the populations continued to die after the observation period.

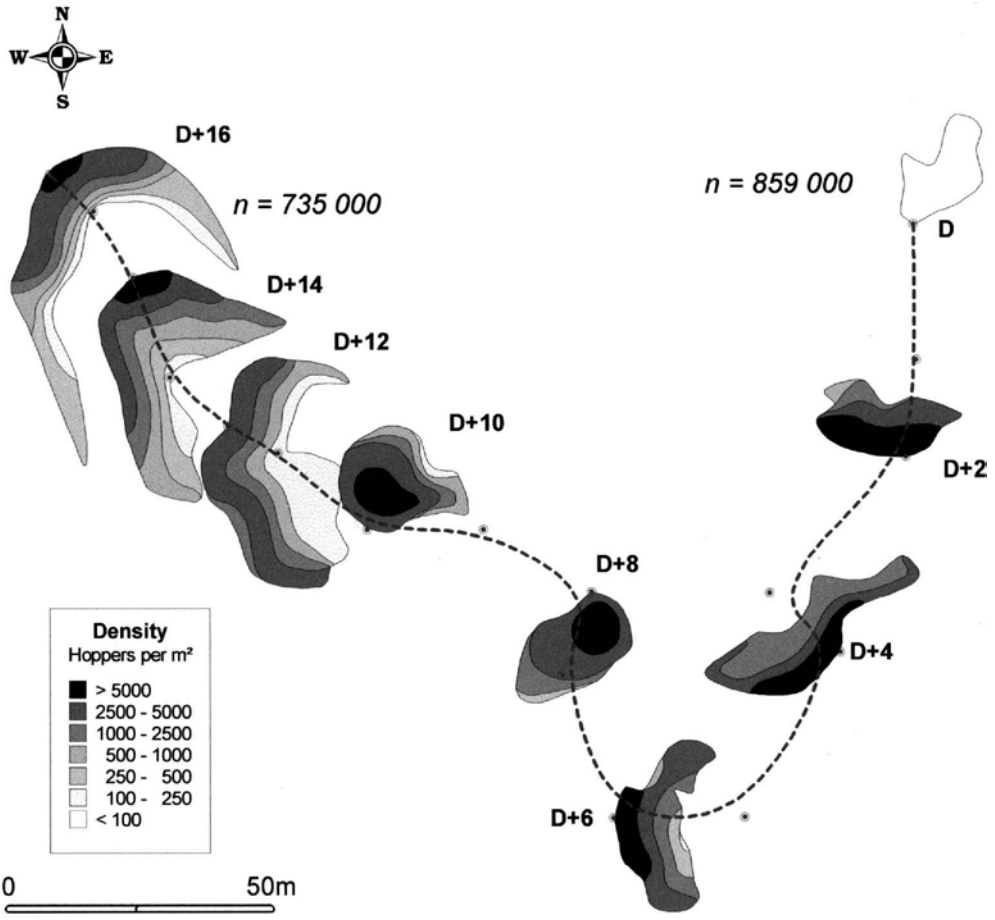

FIGURE 4. Natural evolution in the field of a control grasshopper band (C2); D, days of observation.

Laboratory Cage Monitoring

There was a clear effect of the fungus against *R. schistocercoides* nymphs collected from treated bands and monitored in the laboratory. Insects collected 14 h, 3 and 7 days post-treatment of the fungus showed mortality rates of 76.7, 58.7 and 64% and confirmed fungal infection levels of 68.7, 48.7 and 41.3%, respectively (Table 4). For untreated insects, average mortality was 11.3% after 15 days, whereas infection rate due to *M. anisopliae* var. *acridum* was less than 3%.

Total mortality in the treated grasshoppers at 14 h post-application was shown to be significantly different from the control treatment ($P = 0.002$). On the other hand, mortality in the treated grasshoppers at 14 h was not significantly different from either 3 days ($P = 0.076$) or 7 days post-application ($P = 0.141$). The 14 h post-application treatment was not significantly different from the three days post-application treatment ($P = 0.068$) in terms of fungal infection rates, but was different from data observed at 7 days post-application ($P = 0.037$).

The band treated with low viability conidia plus sublethal dosage of fenitrothion was neither reduced in population size nor presented detectable changes in grasshopper behaviour during the time of assessment (8 days).

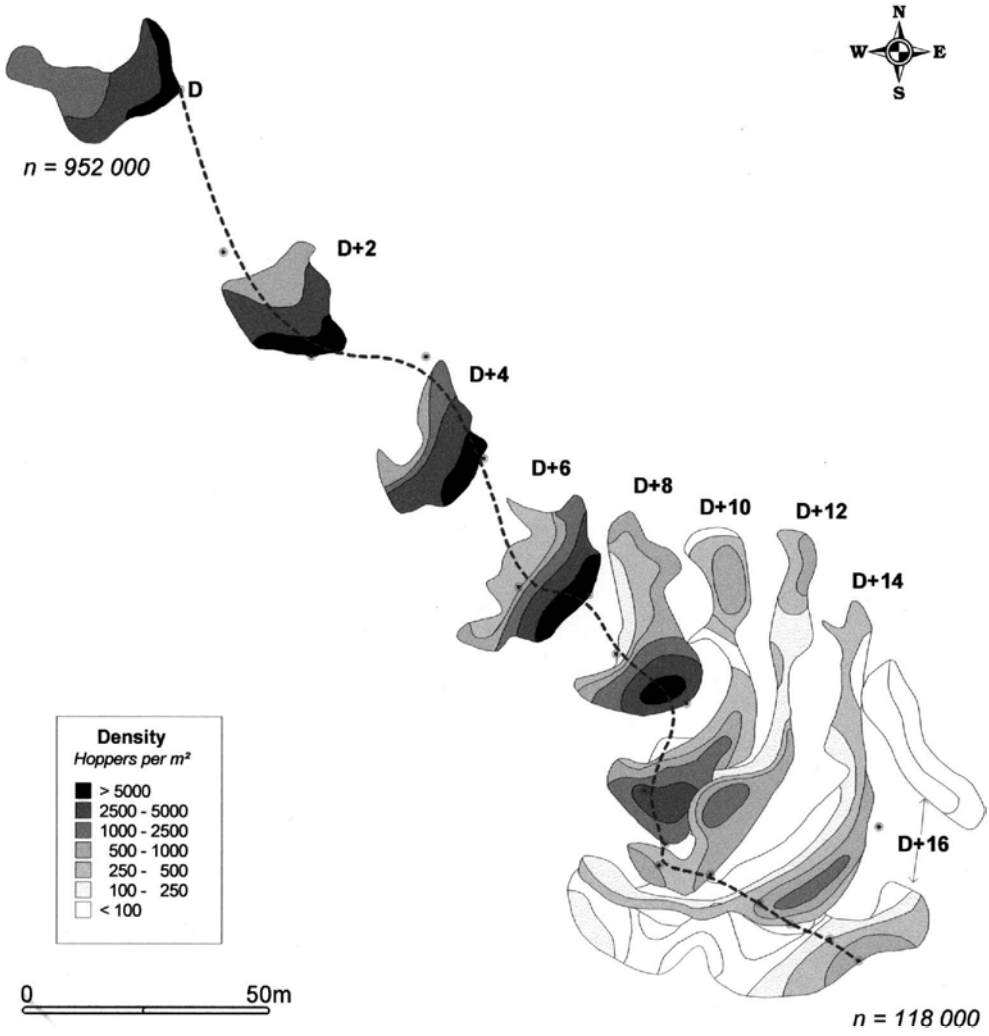

FIGURE 5. Natural evolution in the field of a treated hopper band (F3); D, days of observation.

DISCUSSION

The result of this study, which was carried out under natural conditions, clearly demonstrated the high efficacy of the *M. anisopliae* var. *acridum*-based mycopesticide in controlling *R. schistocercoides* grasshopper populations. This effect was clearly noted in the field 10 days after treatment. The dose used (2×10^{13} conidia ha^{-1}) is quite high, but we considered it acceptable as a first experiment. Other trials with lower doses are under way.

The assessment method used enabled us to characterize quantitatively highly-gregarious grasshoppers, which are the best targets for control operations. These bands were separately monitored for more than 2 weeks during their erratic movements through *campo-cerrado* type natural vegetation zones (shrub and tree savannas). This method was found to be very efficient for demonstrating the effects of the mycopesticide product, despite the fact that it was difficult to obtain accurate measurements of high density grasshopper populations. The highest grasshopper densities, which were pooled into a '5000 m^{-2} and more' category,

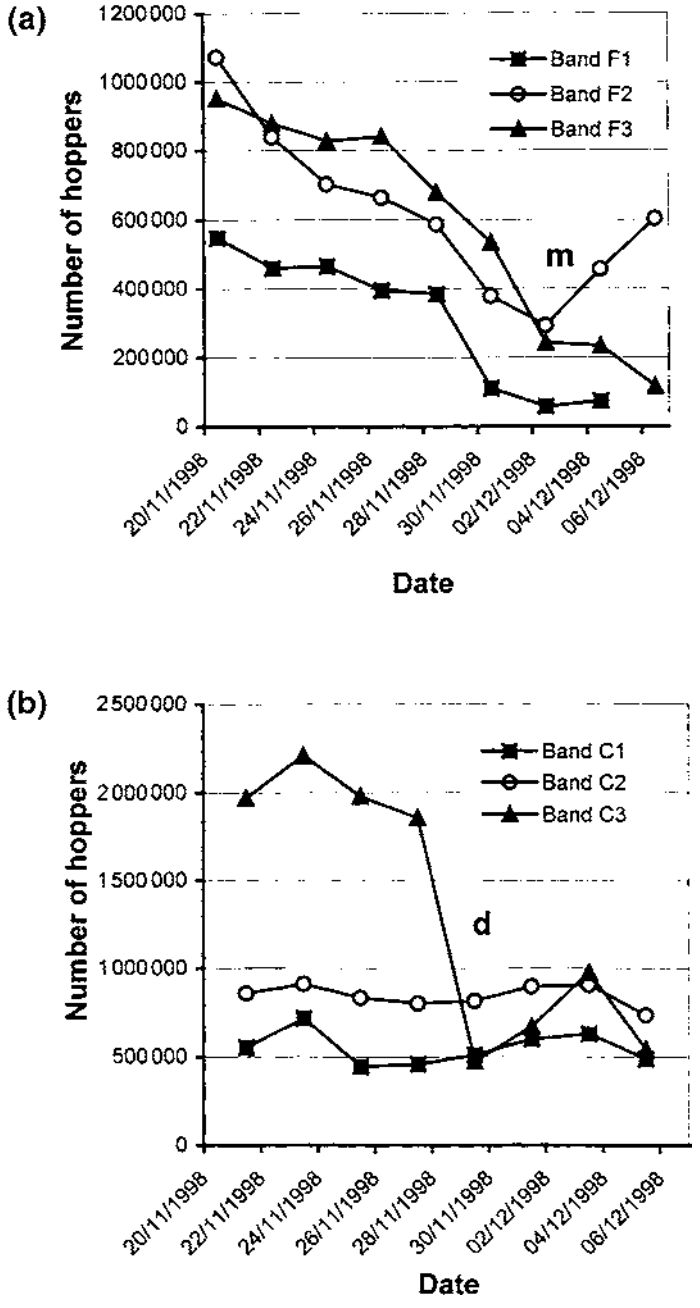

FIGURE 6. Evolution of hopper numbers in: (a) treated and (b) untreated bands. T, date of treatment (20 November); m, merger of band F2 with another band; d, division of the hopper band C3; mycoinsecticide sprayed on 20 November 1998.

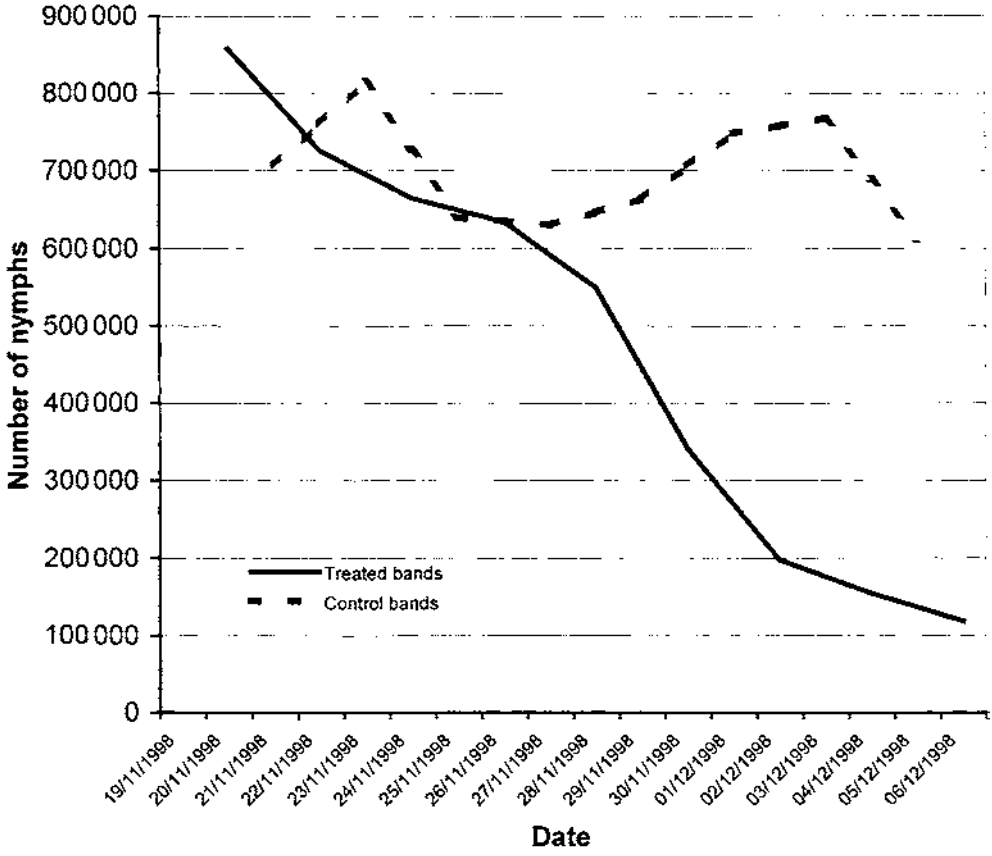

FIGURE 7. Evolution of grasshopper numbers: comparison of treated and untreated bands. Treated bands: Band F3 was not considered after 3 December (fusion with another band). Control bands C1 and C2 were considered; band C3 divided during the experimental period after 9 days; mycoinsecticide sprayed on 20 November 1998.

TABLE 3. Mortality of *R. schistocercoides* nymphs sprayed with *M. anisopliae* var. *acridum* in the field

	Hopper bands	Mortality (%)	Days post-spraying	Remarks
Treated	F1	88.6	14	Hopper band merged with another one at day 14
	F2	72.7	12	
	F3	87.6	14	
Control	C1	11.8	14	Band divided at day 8 and was not followed further
	C2	14.4	14	
	C3	—	—	

were undoubtedly underestimated. This means that mortality rates associated with the mycopesticide treatments were probably underestimated, i.e. higher than the 88% recorded in the study.

The control band population variations seems to have been mainly due to inaccuracies related to the grasshopper density assessment method. We did not observe any significant

TABLE 4. Mortality of *R. schistocercoides* nymphs sprayed with *M. anisopliae* var. *acridum* in the field and kept in cages in the laboratory

Treatments	Days under observation	Mortality (± SEM)	
		Total (%)	Mycosis (%)
14 h post-spraying	15	76.7 ± 9.61	68.7 ± 10.48
3 days post-spraying	13	58.7 ± 3.33	48.7 ± 2.40
7 days post-spraying	9	64.0 ± 3.46	41.3 ± 4.37
Control (= untreated)	15	11.3 ± 5.81	2.7 ± 1.76

decline in the control band populations due to natural mortality between 20 November and 6 December. A dynamic population model (Miranda *et al*., 1996) has been developed based on a daily natural mortality rate of 0.86% during grasshopper development. Assuming there was an initial population of 1 million grasshoppers on 20 November, the surviving population on 6 December would be 870 929 grasshoppers according to this model. The method used in the field is certainly not sufficiently accurate to estimate such a population decrease.

The mortality rate of insects collected in the field 14 h post-treatment and incubated in cages in the laboratory (76.7%) was close to mortality rates reported for mycoinsecticides in other grasshopper species. For example, *M. anisopliae* var. *acridum* caused 90% mortality of desert locust, *Schistocerca gregaria*, populations in Mauritania (Lomer *et al*., 1997) and > 90% on *Phaulacridium vittatum* in Australia Milner (1997); *B. bassiana* caused 67.5% mortality on *Locusta migratoria* when insects were treated with a flowable oil formulation (Delgado *et al*., 1997). In all cases, samples were collected not later than 24 h after treatment.

As expected, mortality levels of insects collected three and seven days post-application tended to be lower than the recorded value for insects collected 14 h post-application. In part, this may be attributed to factors such as the reduced period of observation in samples taken at three and seven days, or inadequate sampling. Sampling was accomplished by collecting insects at the 'front line', region of the band where the grasshopper density was higher. However, many studies have shown that food intake and mobility are hampered by internal development of a fungal pathogen, as early as three days following inoculation of grasshoppers (Seyoum *et al*., 1994; Faria *et al*., 1999). It is likely that infected insects were not able to follow uninfected nymphs or nymphs that received a lower conidial dose on the cuticle and, therefore, sampling was biased against nymphs receiving a higher conidial dose.

Mortality at 14 h post-application treatment did not significantly differ from mortality at three and seven days post-application treatments. Also, confirmed infection of insects collected at 14 h and 3 days post-application did not differ. This result supports the idea that solar radiation did not play a major role in conidial inactivation in contrast to observations from other studies (Inglis *et al*., 1995; Moore *et al*., 1993; Fargues *et al*., 1988). Indeed, population reduction in the field was over 88% after 14 days, indicating no significant effect of solar radiation on fungal performance.

Fenitrothion is at present the only chemical registered in Brazil for grasshopper control, and association with fungal conidia may be a suitable strategy for reducing time for death of insects. In this experiment, the dosage used was not enough to cause detrimental effects on treated insects. Results recently obtained in our laboratory showed that concentrations up to 430 ppm do not significantly affect the germination of the CG 423 strain (S. Xavier-Santos *et al*. unpublished observations). Field assays aimed to verify the possibility of association are being planned for the near future.

The number of conidia estimated to be deposited on each nymph cuticle was quite variable between replicates (1547 ± 430.4). This value is probably an underestimate, since the protocol used may not allow complete removal of conidia adhered to the cuticle, and during the process some volume of kerosene containing conidia is lost. However, this procedure allows a rapid estimate, and modifications such as the use of a larger number of nymphs and a better washing protocol could result in more reliable data.

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